PATENT
ATTORNEY DOCKET NO. 50304/063001

Certificate of Mailing: Date of Deposit: October 18, 2005

I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated above and is addressed to Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Liane M. Malcos
Printed name of person mailing correspondence

Liane M. Malcos
Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	SAINT-REMY et al.	Art Unit:	1644
Serial No.:	10/044,569	Examiner:	Maher M. Haddad
Filed:	January 11, 2002	Customer No.:	21559
Title:	METHOD AND PHARMACEUTICAL COMPOSITION FOR PREVENTING AND/OR TREATING SYSTEMIC INFLAMMATORY RESPONSE SYNDROME		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TERM OF DEPOSIT

I, Marc G. Jacquemin, hereby declare:

1. I am a named inventor on the above-identified patent application.

2. Cell line LMBP 5089CB, an original deposit made under the Budapest Treaty with the Belgian Coordinated Collections of Microorganisms (BCCM™), shall be maintained for a term of at least thirty (30) years or five (5) years after the most recent request for the furnishing of a sample of the deposit was received by the BCCM™ or for the enforceable life of the patent for which the deposit was made.

3. Any restrictions on the availability to the public of cell line LMBP 5089CB will be irrevocably removed upon the granting of a patent on this application, with the exception of those restrictions listed in 37 C.F.R. § 1.808(b).

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: August 1, 2005

Marc G. Jacquemin

PATENT
ATTORNEY DOCKET NO. 50304/063001

Certificate of Mailing; Date of Deposit: October 18, 2005

I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated above and is addressed to Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Liane M. Mateos
Printed name of person mailing correspondence

Liane M. Mateos
Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	SAINT-REMY et al.	Art Unit:	1644
Serial No.:	10/044.569	Examiner:	Maher M. Haddad
Filed:	January 11, 2002	Customer No.:	21559
Title:	METHOD AND PHARMACEUTICAL COMPOSITION FOR PREVENTING AND/OR TREATING SYSTEMIC INFLAMMATORY RESPONSE SYNDROME		

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. JEAN-MARIE SAINT-REMY

1. I am a named inventor on the above-referenced patent application.

2. I am a Professor at the University of Leuven and an expert in the field of vascular biology. A copy of my curriculum vitae is attached.

3. I have read and understand the Office Actions mailed April 21, 2004 and December 15, 2004.

4. I present in vivo data from an animal model confirming that an antibody, administered according to the method of the claimed invention, is effective against systemic inflammatory response syndrome (SIRS) such as sepsis.

5. The model that was used, i.e., the induction of sepsis by a single bolus injection of lipopolysaccharide ("LPS") in mice, is a well-established animal model for studying septic shock symptoms and testing potential therapeutic agents in septic shock.

6. In this experiment, we used both the KRIX-1 antibody and a deglycosylated form thereof. The antibody KRIX-1 is produced by the cell line named KRIX 1, which, as detailed in the patent application, was deposited with the Belgian Coordinated Collections of Micro-organisms under accession number LMBP 5089CB. As described in the patent specification, the KRIX-1 antibody binds to an epitope in the C1 domain of FVIII and partially inactivates FVIII. To obtain the latter deglycosylated antibody, we modified carbohydrate attachment sites found in the complementarity determining regions of the KRIX-1 antibody. This modified KRIX-1 antibody was named KRIX-1Q, and was found to retain the binding affinity to the antigen of the KRIX-1 antibody.

7. Preliminary experiments indicated that a single intraperitoneal (IP) injection of 400 µg LPS in wildtype BALB/c mice resulted in a 80% mortality rate within 2 days. In a first experiment using the antibodies, four groups of BALB/c mice (n=8 in each group) were therefore treated by a single IP injection of antibody KRIX-1 or of its deglycosylated form (KRIX-1Q) prior to administration of 400 µg LPS. Mouse survival was followed over time. The results on the prevention and/or treatment of sepsis in this experiment are illustrated in Figure 1A. This Figure shows that all mice survived endotoxin-mediated shock upon treatment with 3 or 30 µg of KRIX-1 or 3 µg of KRIX-1Q. A significant improvement of survival rate was also observed at a dose of 0.3 µg KRIX-1.

8. In another experiment, wildtype C57B1/6 mice were injected with KRIX-1Q (30 µg, 3 µg, or 0.3 µg), a sham IgG4 antibody (AK6A3), or buffer. Thirty minutes later a single IP injection of 400 µg LPS was administered. Survival of the mice was subsequently monitored. When mice were administered the KRIX-1Q antibody an anti-sepsis response was observed. In particular, it was observed that the KRIX-1Q antibody can be administered in high dosages without occurrence of shock as a result of pro-inflammatory and anti-inflammatory compensatory responses as observed with complete inhibition of FVIII. The effectiveness against sepsis is illustrated in Figure 1B. This Figure shows a statistically significant death prevention with 30 µg of KRIX-1Q compared to mice receiving either the sham IgG4 (AK6A3) ($p<0.03$) antibody or no antibody (i.e., buffer).

9. The results illustrated in Figure 1A and 1B clearly demonstrate that the KRIX-1 and KRIX-1Q antibodies are effective against sepsis in the mouse model.

10. To further demonstrate that partially inhibitory antibodies directed against the C1 domain of FVIII are readily obtained following the methods described in the specification of our patent application, we present the following data in connection with the antibody named RHD5.

11. In general, a human lymphoblastoid cell line, named RHD5, was derived by immortalization of B lymphocytes from a patient with acquired hemophilia, as described in the specification. These B cells were then transformed by infection with Epstein-Barr virus as follows. Briefly, 10^7 peripheral blood mononuclear cells were resuspended in 2 mL culture medium and incubated for 2 hours at 37°C with 200 µL Epstein-Barr virus supernatant (B95-8 strain). Cells were then seeded at 5,000 cells/well in 96-well microtiter plates (Nunc) containing feeder cells (3T6-TRAP cells irradiated with 7,000 rads). One hundred fifty microliters of culture supernatant was replaced every week by fresh culture medium.

12. After 6 weeks, culture supernatants were tested in an enzyme-linked immunosorbent assay (ELISA) for the presence of anti-FVIII antibodies. Positive cell lines were transferred to 24-well plates and immediately cloned at 60 cells per 96-well plate without feeder cells. One clone, producing an antibody called RHD5, was selected. The antibody present in the culture supernatant was purified by adsorption on HiTRAP protein A (Pharmacia), as described in the specification.

13. The fact that RHD5 binds to the C1 domain of FVIII, similar to KRIX-1 was confirmed by immunoreactivity to FVIII fragments corresponding to the C1 domain of FVIII.

14. Inhibitory activity or RHD5 was assessed in a Bethesda assay. RHD5 inhibited only partially FVIII activity up to the highest concentration tested. In a Bethesda assay performed by mixing one volume of antibody at 200 microgram/mL or of control buffer with one volume of plasma, the residual FVIII levels were 7.0 ± 0.2 and 251.9 ± 18.8 ng/mL, respectively (mean ± SD of triplicates). RHD5 (at a final concentration of 100 µg/mL) inhibited FVIII by at least 97%. Similarly, in a Bethesda assay performed by mixing one volume of RHD5 antibody at 200 microgram/mL or of control buffer with one volume of full length recombinant FVIII (Recombinate^{R}, Baxter), the residual FVIII levels were 8.0 ± 0.2 and 399.7 ± 18.8 ng/mL, respectively (mean ± SD of triplicates). The inhibition of FVIII activity reached at a final concentration of RHD5 of 100 microgram/mL was therefore 98%. A dose response curve of plasma FVIII inhibition by RHD5 is shown in Figure 2.

15. The ability of KRIX-1 to compete with RHD5 for FVIII binding was also tested in ELISA. Polystyre microtitration plates were incubated overnight at 4°C with 50 µL RHD5 at 2 microgram/mL in phosphate buffered saline (PBS). The plates were next washed 4 times with PBS-Tween. Biotinylated recombinant FVIII (0.5 microgram/mL) in Tris-BSA-Tween was mixed with RHD5 or KRIX-1 at various concentrations before addition to RHD5 coated plates. After a two hour incubation period at 4°C, the plates

were washed 4 times and bound biotinylated FVIII was detected by addition of avidine peroxidase (Sigma) at 1 microgram/mL. After 30 minutes at room temperature (RT), the plates were washed again and supplemented with 100 µL OPD. The resulting OD was read at 490 nm in a Emax Microplate Reader (Molecular Devices, Menlo Park, CA). Biotinylated FVIII used in the above experiment was prepared by incubating recombinant FVIII (100 microgram/mL) dialysed in Hepes buffer (Hepes 10 mM, NaCl 0,15 M, $CaCl_2$ 10 mM, pH 8.5) with sulfo-NHS-LC-biotin (Pierce) at 1 microgram/mL for 2 hours at RT. The preparation was then dialysed against Hepes buffer and stored and –80°C.

16. As shown in Figure 3, KRIX-1 completely prevented FVIII binding to RHD5. These data confirm that RHD5, like KRIX-1, is directed against the C1 domain of FVIII.

17. I note that these data support the fact that antibodies such as KRIX-1 and RHD5 directed against the C1 domain of factor VIII and capable of partially inhibiting FVIII are indicative of results which can be obtained following the methods described in the application.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: OCTOBER 13 2005

Dr. Jean-Marie Saint-Remy

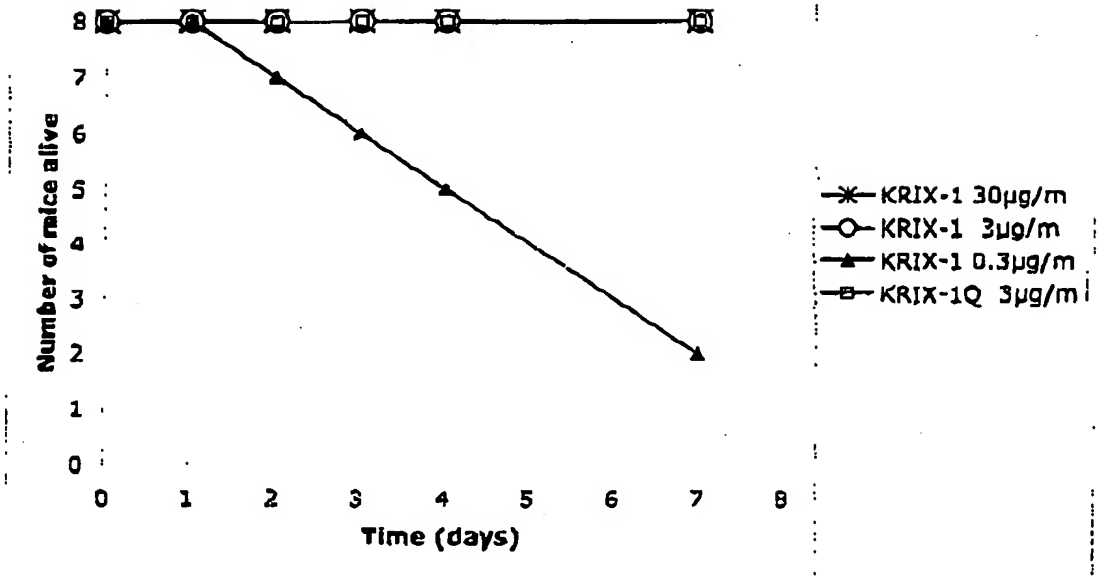

Figure 1A: Survival in a septic shock model of mice upon co-administration of LPS with partial inhibitory antibodies against Factor VIII

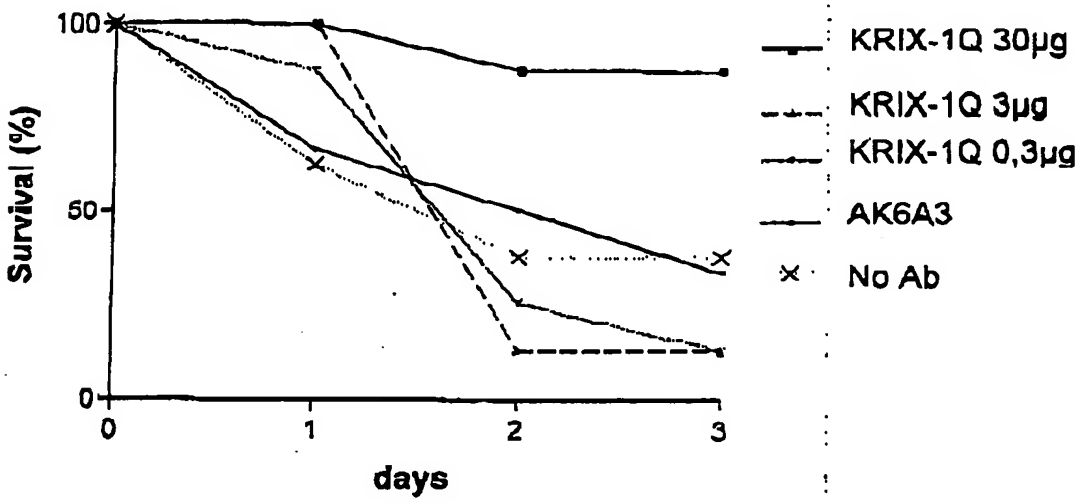

Figure 1B: Survival in a septic shock model of mice pretreated with partial inhibitory antibodies against Factor VIII.

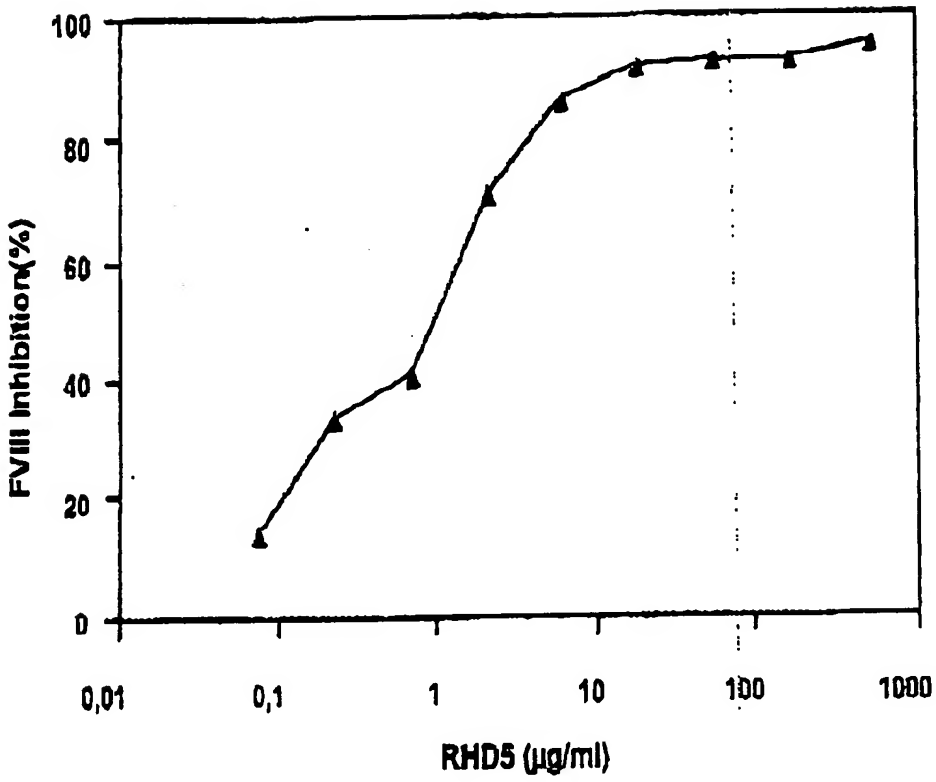

Figure 2: Dose response curve of plasma FVIII inhibition by RHD5

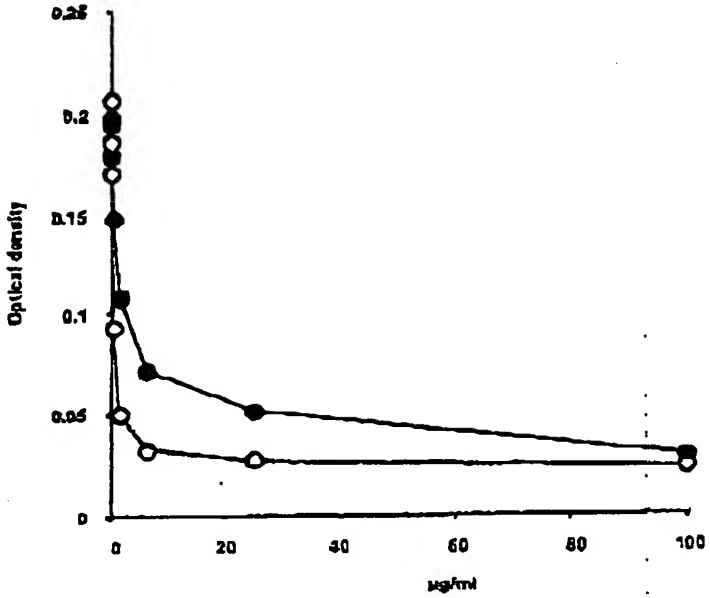

Figure 3: Competition of RHD5 and Krix-1 for the binding to C1 domain of FVIII. Different concentrations of RHD5 (closed symbols) or Krix-1 (open symbols) were mixed with rFVIII before addition to RHD5 coated plates. The plates were then incubated for 2 hours at 4°C and the binding of FVIII was detected by the addition of avidine peroxidase and OPD.

Jean-Marie R. SAINT-REMY

Curriculum vitae, June 2005

Personal data

Address: Rue du Lambais, 79
1390 Grez-Doiceau
Belgium
Tel: +32.10.84.14.23
Fax: +32.10.84.29.43

Office: Center for Molecular and Vascular Biology
University of Leuven, Campus Gasthuisberg
Herestraat 49
B-3000 Leuven, Belgium
Tel: +32-16-345.791
Fax: +32-16-345.990
e-mail jeanmarie.saint-remy@med.kuleuven.ac.be

Education

1974	Doctor in Medicine (MD), UCL, Belgium
1979	Board certified Specialist in Internal Medicine, UCL, Belgium
1982	PhD in Immunology, University of London (UK)
1992	Agregation for Higher Education in Medicine, UCL, Belgium

Appointments within the University of Leuven

1995-1996	Research Associate
1996-1999	Docent (Assistant Professor)
1999- present	Hoofddocent (Associate Professor)

Academic Appointments outside the University of Leuven

1982-1989	Senior Investigator, Institute of Cellular and Molecular Pathology Université de Louvain, Brussels, Belgium
1989-1995	Research Director, Allergy and Clinical Immunology Unit, Université de Louvain, Brussels, Belgium

Other Activities

1993-2002	President of the Belgian Society for Allergy and Clinical Immunology
1996-	Consultant, Allergy and Clinical Immunology, Institut Edith Cavell, Brussels, Belgium

Awards and Honors

1980-81	Fellowship of the International Institute for Molecular and Cellular Pathology (ICP, Brussels, Belgium)
1983-84	Pharmacia Award for Allergy and Clinical Immunology
1989	de Hovre Foundation Award for Immunology
2003-2005	Bayer International Award for Haemophilia basic research
2005-2007	Bayer International Award for Haemophilia special projects

Membership in Scientific Organizations

1980	Belgian Society for Allergy and Clinical Immunology
1992	British Society for Allergy and Clinical Immunology
1988	European Academy for Allergy and Clinical Immunology
1988	International Association for Allergy and Clinical Immunology
1993	Belgian Society for Thrombosis and Haemostasis
1994	Société belge d'Oto-Rhino-Laryngologie
1994	European Ligand Association
1997	American Society of Hematology
1999	International Society for Thrombosis and Haemostasis
2000	Collegium Internationale Allergologicum

Publications

Author on over 100 papers published in international peer-reviewed journals, of which a selection is provided herunder.

1. Saint-Remy JM, Lacroix-Desmazes S, Oldenburg J. Inhibitors in haemophilia: pathophysiology. Haemophilia. 2004 Oct;10 Suppl 4:146-51. Review.

2. Pipe SW, Saint-Remy JM, Walsh CE. New high-technology products for the treatment of haemophilia. Haemophilia. 2004 Oct;10 Suppl 4:55-63. Review.

3. Jacquemin M, Saint-Remy JM. The use of antibodies to coagulation factors for anticoagulant therapy. Curr Med Chem. 2004 Sep;11(17):2291-6. Review.

4. Jacquemin MG, Saint-Remy JM. Factor VIII alloantibodies in hemophilia. Curr Opin Hematol. 2004 May;11(3):146-50. Review.

5. Gilles JG, Grailly SC, De Maeyer M, Jacquemin MG, VanderElst LP, Saint-Remy JM. In vivo neutralization of a C2 domain-specific human anti-Factor VIII inhibitor by an anti-idiotypic antibody. Blood. 2004 Apr 1;103(7):2617-23. Epub 2003 Dec 11.

6. d'Oiron R, Lavergne JM, Lavend'homme R, Benhida A, Bordet JC, Negrier C, Peerlinck K, Vermylen J, Saint-Remy JM, Jacquemin M. Deletion of alanine 2201 in the FVIII C2 domain results in mild hemophilia A by impairing FVIII binding to VWF and

phospholipids and destroys a major FVIII antigenic determinant involved in inhibitor development. Blood. 2004 Jan 1;103(1):155-7. Epub 2003 Sep 11.

7. Dewerchin M, Van der Elst L, Singh I, Grailly S, Saint-Remy JM, Collen D, Jacquemin M. Inhibition of factor VIII with a partially inhibitory human recombinant monoclonal antibody prevents thrombotic events in a transgenic model of type II HBS antithrombin deficiency in mice. J Thromb Haemost. 2004 Jan;2(1):77-84.

8. Janssens W, Carlier V, Wu B, VanderElst L, Jacquemin MG, Saint-Remy JM. CD4+CD25+ T cells lyse antigen-presenting B cells by Fas-Fas ligand interaction in an epitope-specific manner. J Immunol. 2003 Nov 1;171(9):4604-12.

9. Raut S, Villard S, Grailly S, Gilles JG, Granier C, Saint-Remy JM, Barrowcliffe TW. Anti-heavy-chain monoclonal antibodies directed to the acidic regions of the factor VIII molecule inhibit the binding of factor VIII to phospholipids and von Willebrand factor. Thromb Haemost. 2003 Sep;90(3):385-97.

10. Villard S, Lacroix-Desmazes S, Kieber-Emmons T, Piquer D, Grailly S,Benhida A, Kaveri SV, Saint-Remy JM, Granier C. Peptide decoys selected by phage display block in vitro and in vivo activity of a human anti-FVIII inhibitor. Blood. 2003 Aug 1;102(3):949-52. Epub 2003 Apr 3.

11. Jacquemin M, De Maeyer M, D'Oiron R, Lavend'Homme R, Peerlinck K, Saint-Remy JM. Molecular mechanisms of mild and moderate hemophilia A.J Thromb Haemost. 2003 Mar;1(3):456-63. Review. Erratum in. J Thromb Haemost. 2003 Dec;1(12):2722.

12. Jacquemin M, Vantomme V, Buhot C, Lavend'homme R, Burny W, Demotte N, Chaux P, Peerlinck K, Vermylen J, Maillere B, van der Bruggen P, Saint-Remy JM. CD4+ T-cell clones specific for wild-type factor VIII: a molecular mechanism responsible for a higher incidence of inhibitor formation in mild/moderate hemophilia A. Blood. 2003 Feb 15;101(4):1351-8. Epub 2002 Oct 17.

13. Janssens W, Chuah MK, Naldini L, Follenzi A, Collen D, Saint-Remy JM, VandenDriessche T. Efficiency of onco-retroviral and lentiviral gene transfer into primary mouse and human B-lymphocytes is pseudotype dependent. Hum Gene Ther. 2003 Feb 10;14(3):263-76.

14. Kallas A, Pooga M, Benhida A, Jacquemin M, Saint-Remy JM. Epitope specificity of anti-FVIII antibodies during immune tolerance therapy with factor VIII preparation containing von Willebrand factor. Thromb Res. 2002 Sep 15;107(6):291-302.

15. Wu B, Elst LV, Carlier V, Jacquemin MG, Saint-Remy JM. The Dermatophagoides pteronyssinus group 2 allergen contains a universally immunogenic T cell epitope. J Immunol. 2002 Sep 1;169(5):2430-5.

16. Behrmann M, Pasi J, Saint-Remy JM, Kotitschke R, Kloft M. Von Willebrand factor modulates factor VIII immunogenicity: comparative study of different factor VIII concentrates in a haemophilia A mouse model. Thromb Haemost. 2002 Aug;88(2):221-9.

17. Saint-Remy JM. Immunology of factor VIII inhibitors. Semin Thromb Hemost. 2002 Jun;28(3):265-8. Review.

18. Singh I, Smith A, Vanzieleghem B, Collen D, Burnand K, Saint-Remy JM, Jacquemin M. Antithrombotic effects of controlled inhibition of factor VIII with a partially inhibitory human monoclonal antibody in a murine vena cava thrombosis model. Blood. 2002 May 1;99(9):3235-40.

19. Saint-Remy JM. Hemophilia factor VIII therapy. B- and T-cell tolerance from basic concepts to clinical practice. Haematologica. 2000 Oct;85(10 Suppl):93-6. Review.